that TRPM8 channel deactivation is well described by a double exponential time course. The fast and slow deactivation processes are temperature-dependent with enthalpy changes of 27.2 kcalmol-1 and 30.8 kcalmol-1. The overall Q10 for the closing reaction is about 33. A three-tiered allosteric model containing four voltage sensors and four temperature sensors can account for the complex deactivation kinetics and coupling between voltage and temperature sensor activation and channel opening.

184-Plat
TRPM8 is an Ionotropic Testosterone Receptor
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Testosterone is a key steroid hormone in the development of male reproductive tissues and in the regulation of the central nervous system. Rapid signaling mechanism induced by testosterone affects numerous behavioral traits, including sexual behavior, aggressiveness, or fear conditioning. However, currently identified testosterone receptor(s) are not believed to underlie the fast signaling. Here we report that an ion channel from the transient receptor potential family, TRPM8, commonly known as the cold and menthol receptor is the major component of testosterone-induced rapid actions.
TRPM8 is highly expressed in the prostate epithelial cells. Using an immunohistochemistry approach, we detected a colocalization pattern of TRPM8 with endogenous androgens on human prostate tissues obtained from healthy individuals and patients with prostate cancer. Co-immunoprecipitation experiments performed on cultured prostate epithelial cells, prostate cancer cells, and HEK-293 cells stably expressing TRPM8, further confirmed direct binding of testosterone to TRPM8.
Using cultured and primary cell lines and the purified TRPM8 protein we demonstrate that testosterone can directly activate TRPM8 channel at low picomolar range. Specifically, testosterone induced TRPM8 responses in primary human prostate cells, prostate cancer cells PC3, dorsal root ganglion (DRG) neurons, and hippocampus neurons. Picomolar concentrations of testosterone resulted in full openings of the purified TRPM8 channel in planar lipid bilayers. Furthermore, acute applications of testosterone on human skin elicited cooling sensation, implying testosterone-induced activation of TRPM8. Additionally, animal studies showed altered sniffing behavior and increased blood concentration of testosterone in the TRPM8 knockout mice, suggesting the existence of a feedback loop mechanism in the absence of the receptor. Our data demonstrate that testosterone is an endogenous and highly potent agonist of TRPM8, suggesting a role of TRPM8 channels well beyond their well-established function in somatosensory neurons. This discovery may further imply TRPM8 channel function in testosterone-dependent behavioral traits.

Platform: Protein Lipid Interactions I

185-Plat
Experimental and Computational Studies of Pulmonary Surfactant Protein SP-B Interacting with Lipid Bilayers
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Pulmonary lung surfactant protein B (SP-B) is a 79 residue hydrophobic protein, from the Saposin superfamily. Saposin super family proteins share common features including 3 intra-chain disulfide bonds and 4-5 helical regions. SP-B appears to carry out its essential functions in respiration by binding to and modifying the structures of phospholipid bilayers and monolayers at the air-water interface. Due to difficulties arising from SP-B's extreme hydrophobicity, the 3D structure of full SP-B is not yet known. Thus we are using computational methods in combination with solid-state NMR to investigate the structure of SP-B. Our approach is to generate candidate structures via computational methods, predict the 15N spectra for the computed configurations and then compare them to the experimental NMR spectra.
For the computational studies, we employ Mini-b, a construct of SP-B with known structure, and add the rest of residues to make a full length SP-B. We use several positions of SP-B in a POPC bilayer as initial structures. Calculations are carried out using GROMACS, with OPLS-aa, an all-atom force field, as well as with PACE, a hybrid model force field. Simulation methods include Molecular Dynamics (MD) and Replica Exchange Molecular Dynamics (REMD). For the experimental part, we use recombinantly expressed 15N labeled SP-B in mechanically oriented POPC bilayers to collect 1D 15N solid-state NMR spectra.

186-Plat
Creation of Water-Soluble Integral Membrane Proteins using an Engineered Amphipathic Protein "Shield"
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Integral membrane proteins (IMPs) play crucial roles in all cells and represent attractive pharmacological targets. However, functional and structural studies of IMPs are hindered by their hydrophobic nature and the fact that they are generally unstable following extraction from their native membrane environment using detergents. Here, we devised a general strategy for in vivo solubilization of IMPs in a functional conformation without the need for detergents or mutations to the IMP itself. This technique, called SIMPLEx (solubilization of IMPs with high levels of expression), involves creating fusions between an IMP target and truncated apolipoprotein A-I, which serves as an amphipathic proteic "shield" that sequesters the IMP from water and promotes its solubilization.

187-Plat
Validating the Retinal Flip of Rhodopsin using Molecular Dynamics
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Rhodopsin, the mammalian dim light photoreceptor, is the most well-characterized structural model of a G protein-coupled receptor (GPCR). Photoisomerization of its covalently bound chromophore, retinal, triggers rhodopsin activation. Spectroscopic studies of rhodopsin in the dark [1] and Meta I [2] states have definitively shown that the C9- and C13-methyl groups of retinal are oriented towards the extracellular side of the protein. However, the structures of the active Meta II state [3] and a constitutively active triple mutant [4] had a 180° rotation along the long-axis of the retinal polyene chain, leading to an orientation of the C9- and C13-methyl groups towards the cytoplasmic side of the protein. The biophysical significance of this potential flip and its role in the structural transition during activation is still unknown. We employed molecular dynamics simulations to determine the role of the retinal flip in rhodopsin activation. Rhodopsin was modeled starting with the Meta II crystal structure but in the Meta I protonation state, to favor a deactivation transition. Surprisingly, two of our four simulations produce a reverse flip of the polyene chain on the microsecond timescale. This flip is accompanied by the rotation of the Trp265 side chain, which is implicated in a "transmission switch" common to GPCR activation. A decrease of water within the retinal binding pocket is also observed, along with distinct protein hydration features concurrent with the flipping of retinal. These results provide a bridge between spectroscopic and crystallographic studies, showing that it is possible for a retinal flip to occur from Meta I to Meta II state. [1] Salgado (2004) *Biochemistry* 43:12819; [2] Salgado (2006) *JACS* 128:11067; [3] Choe (2011) *Nature* 471:651; [4] Deupi (2012) *PNAS* 109:119.

188-Plat
HIV gp41-Antibody Interaction at the Viral Membrane Interface Defined by EPR Spectroscopy
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HIV enters human T cells through the fusion of viral and host-cell membranes. This fusion process is mediated by a surface protein, gp41, and the platform provided by the cholesterol-rich viral membrane. The membrane proximal ectodomain region (MPER) of gp41 plays a critical role in this fusion process and is a major target of anti-gp41 antibodies and vaccine design. Here, EPR and NMR techniques were used to define MPER structure on the membrane, and how neutralizing anti-gp41 antibodies recognize their membrane-immersed epitopes and disrupt a hinge-related function of the MPER. The analyses of several HIV-1 clade B and clade C MPERs revealed a structurally conserved pair of helices immersed in the viral membrane separated by a flexible hinge, which include critical helix capping residues. Double alanine mutations of the capping residues result in an altered hinge structure with a deeper lipid-buried MPER middle region, as well as